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Properties of Protein Powders from Arrowtooth Flounder (*Atheresthes stomias*) and Herring (*Clupea harengus*) Byproducts

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Functional, nutritional, and thermal properties of freeze-dried protein powders (FPP) from whole herring (WHP), herring body (HBP), herring head (HHP), herring gonad (HGP), and arrowtooth flounder fillets (AFP) were evaluated. The FPP samples have desirable nutritional and functional properties and contained 63−81.4% protein. All FPP samples had desirable essential amino acid profiles and mineral contents. The emulsifying and fat adsorption capacities of all FPP samples were higher than those of soy protein concentrate. The emulsifying stability of WHP was lower than that of egg albumin but greater than that of soy protein concentrate. Thermal stability of the FPP samples is in the following order: HGP > HBP > WHP > HHP > AFP.

KEYWORDS: Arrowtooth flounder; fish byproducts; fish protein powders; functional properties; herring; thermal analysis

INTRODUCTION

It has been estimated that >1 million metric tons (mt) of fish-processing byproducts is produced annually in Alaska (*1*). These byproducts are currently processed into fish meal and oil or returned to the sea, even though they could be used to make food-grade protein powders and other functional ingredients. A total of 32,509 mt of herring (*Clupea harengus*) was harvested in Alaska in 2000 (*1*). The majority of herring harvested in Alaska is for herring roe, a valuable commodity known as "kazunoko" in the Japanese market. The male and spent female herring are often made into in fish meal.

Arrowtooth flounder (*Atheresthes stomias*) is an underutilized flatfish abundantly found in the Alaska waters. In the Bering Sea and Aleutian Islands, the National Marine Fisheries Service has estimated an annual exploitable biomass of the arrowtooth flounder at 576,000 mt. In 2002, only 32,610 mt was caught, of which 18,200 mt was discarded (*2*). The small amount of arrowtooth flounder harvested is usually made into fish meal. The major obstacle to utilizing arrowtooth flounder for human consumption is the presence of proteolytic enzymes that soften the flesh during cooking, making it unpalatable to many consumers (*3*). Despite this problem, a small market exists for fillets marketed as turbot or French sole. Furthermore, a process has been developed to make surimi from arrowtooth flounder, but the product has been limited to a mixture of arrowtooth and pollock surimi (*4*).

Many protein-rich seafood byproducts have a range of dynamic properties (*5*) and can potentially be used in foods as binders, emulsifiers, and gelling agents. Soy and milk proteins are widely used in many segments of the food industry, whereas amino acids and peptides are gaining much use in energy drinks and other applications (*6*). Fish are regarded as an excellent source of high-quality protein, particularly of the essential amino acids lysine and methionine. Due to a lack of a suitable purification process to preserve protein functionality, fish protein is noticeably absent in the rapidly growing protein ingredient and health markets. The objective of this study was to develop and characterize the quality of freeze-dried fish protein powders (FPP), prepared from arrowtooth flounder, whole herring, herring body, herring head, and herring gonad, that can potentially be used as functional ingredients and nutritional supplements.

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MATERIALS AND METHODS

Materials. Fresh arrowtooth flounder (*A. stomias*) and herring (*C. harengus*) were obtained from commercial fish-processing plants in Kodiak, AK. Fish were immediately processed. Fish parts, that is, whole herring (WH), herring body (HB) (only the head and gonads were removed), herring head (HH), herring gonads (HG, testes), and arrowtooth flounder fillets (AF), were vacuum packaged and stored at −40 °C until further processed into FPP.

Preparation of FPP Samples. WH, HB, HH, HG, and AF were thawed at 4 °C overnight. Thawed samples were ground in a Hobart grinder (K5SS, Hobart Corp., Troy, OH) through a 7-cm-diameter plate having 12-mm-diameter openings and subsequently ground through a plate with 6-mm-diameter openings. Protein extraction was done according to the method of Sathivel et al. (*7*). A 500 g portion of each ground fish part was mixed with an equal volume of distilled water and homogenized in a Waring blender (Waring Products Division, New Hartford, CT) for 2 min. The mixture was continuously stirred for 60 min at 85 °C. During heating, fat cells were ruptured, releasing oil into the liquid phase. The heated suspension was centrifuged at 2560*g* for 15 min, resulting in three separate phases: the semisolid phase at the bottom containing insoluble protein, bone, and skin; the heavy liquid phase in the middle containing soluble proteins, and the light liquid phase at the top containing crude lipids. The heavy liquid middle layer was separated, collected, and freeze-dried. The resulting FPP was placed in vacuum bags and stored at 4 °C until analyzed.

Proximate Composition. The FPP samples were analyzed in triplicate for moisture and ash contents using AOAC standard methods 930.15 and 942.05, respectively (*8*). The fat content was determined in triplicate using the Leco FA-100 analyzer (LECO Corp., St. Joseph, MI). The nitrogen content was determined in triplicate using the Leco FP-2000 nitrogen analyzer. The protein content was calculated as percent nitrogen times 6.25. The yield was calculated by determining the weight of FPP as a percentage of the total wet weight of raw material used (*9*):

$$\text{yield } \% = \frac{\text{wt of FPP (g)} \times 100}{\text{raw material (g)}}$$

Amino Acid Analysis. Amino acid analysis was conducted by the AAA Service Laboratory Inc., Boring, OR. All FPP samples were hydrolyzed with 6 N HCl and 2% phenol at 110 °C for 22 h. The cysteine content was determined after performic acid oxidation. Amino acids were quantified using the Beckman 6300 sodium hydrolysate method with postcolumn ninhydrin derivatization. Tryptophan content was not determined.

Mineral Analysis. The mineral content of all FPP samples was determined in triplicate by the acid digestion method involving microwave technology (CEM microwave, MDS-2000, CEM Corp., Matthews, NC). A 0.5 g FPP sample was placed in a vessel, and 6 mL of HNO_3 was added. The sealed vessel was heated until digestion had been completed, and then the sample was cooled for 5 min. The inductively coupled argon plasma (ICAP) machine (model CIROS, Spectro Analytical Instruments, Kleve, Germany) was used to analyze the mineral content.

Color and SDS-PAGE Electrophoresis. The color of the FPP samples was determined using a Minolta Chromameter (model CR-300, Minolta Co., Ltd., Osaka, Japan) and reported as L^*, a^*, and b^*. The SDS-Tricine/polyacrylaminde gel electrophoresis system was used with a Photodyne Foto/Force 300 apparatus under reducing conditions according to the method of Schagger and Von Jagow (*10*). Precast 10−20% Tricine gels (Novel Inc.) were used, and molecular mass standards were purchased from Sigma-Aldrich. The protein bands were visualized from the gels stained with Coomassie blue.

Functional Properties. Three separate experiments for each FPP sample were conducted, and the results were reported on a protein content basis. Purified egg albumin containing 84% protein (J. T. Baker Inc., Phillipsburg, NJ) and soy protein concentrate (SPC) powder containing 66% protein (Central Soya Inc., Fort Wayne, IN) were used as protein references.

Nitrogen solubility was determined following the procedure of Morr et al. (*11*). Five hundred milligrams of each FPP sample was dispersed in 50 mL of 0.1 M NaCl at pH 7.0. The solution was stirred for 1 h at 25 °C and centrifuged at 2560*g* for 30 min. The supernatant was analyzed for a nitrogen content using a Leco FP-2000 nitrogen analyzer. The solubility of FPP samples, defined as the amount of soluble nitrogen from the total nitrogen, was calculated as

$$\text{N solubility } (\%) = \frac{\text{supernatant N concentration}}{\text{sample N concentration}} \times 100$$

Emulsifying capacity (EC) was measured by an oil titration method (*12*). Two hundred milligrams of each FPP sample was dissolved in 20 mL of a 0.1 M NaCl solution in a tared 400 mL beaker. A motorized stirrer of a homogenizer (model 6-105-AF, Virtis Co., Gardiner, NY) was immersed in the solution in the beaker. A separatory funnel filled with 100% pure soybean oil (Hunt-Wesson Inc., Fullerton, CA) was placed above the beaker. A pair of electrodes connected to a multimeter (True RMS multimeter, John Fluke Co., Everett, WA) was immersed in the solution to measure the electrical resistance (in ohms) of the emulsion. The solution was first stirred at 60% output of a 120 V rheostat for 20 s to make a homogenized solution and to get a constant resistance reading. The output was then increased to 100%, and the oil was immediately dispensed from the separatory funnel into the beaker at 0.5 mL/s, generating an oil-in-water emulsion at 25 °C. A sudden increase in resistance was observed when the oil capacity of the FPP emulsion reached a maximum value and the emulsion collapsed to form a water-in-oil emulsion. At that point, oil delivery was stopped and the oil volume measured by weighing the beaker and calculating the quantity in milliliters by correcting for oil density (0.9112 g/mL). EC was expressed as milliliters of emulsified oil per 200 mg of FPP.

Emulsifying stability (ES) was evaluated according to the method of Yatsumatsu et al. (*13*). Five hundred milligrams of each FPP sample was transferred into a 250 mL beaker and dissolved in 50 mL of 0.1 M NaCl, and then 50 mL of soybean oil was added. The homogenizer equipped with a motorized stirrer driven by the rheostat was immersed in the mixture and operated for 2 min at 100% output at 120 V to make an emulsion. From the emulsion, three 25-mL aliquots were immediately taken and transferred into three 25-mL graduated cylinders. The emulsions were allowed to stand for 15 min at 25 °C, and then the milliliters of aqueous volume to total volume was read. ES (%) was calculated as [(total volume − aqueous volume)/total volume] × 100.

The fat absorption capacity (FA) of the FPP samples was determined by placing 500 mg of each FPP sample into a 50-mL centrifugal tube and adding 10 mL of soybean oil (*14*). The sample was thoroughly mixed with a small steel spatula, kept for 30 min at 25 °C with intermittent mixing every 10 min, and then centrifuged at 2560*g* for 25 min. Free oil was then decanted, and the fat absorption of the sample was determined from the weight difference. FA was expressed in terms of milliliters of fat absorbed by 1 g of FPP.

Differential Scanning Calorimetry (DSC). The triplicate experiments were conducted using a differential scanning calorimeter (model DSC 2920, TA Instruments, New Castle, DE). Approximately 0.5−1 mg of each FPP sample was placed in the aluminum sample vessel. The sample vessel was then placed on the sample platform, and an empty aluminum vessel was placed on the reference platform. To determine the phase transition of the FPP samples, a linear heating rate of 5 °C/min over a temperature range of 75−100 °C was used. The thermogram peak was used to provide an estimate of enthalpy (ΔH). From the thermogram peak, a baseline was constructed, the area under the DSC transition curve was measured, and the estimated total denaturation enthalpy (ΔH_{total}) was calculated using the TA Instrument software.

Thermogravimetric Analysis (TG). The thermal stability of each FPP sample was analyzed using a thermogravimetric analyzer (Hi-Res Modulated TGA 2950, TA Instruments). Approximately 0.5−1 mg of each FPP sample was placed on an aluminum pan, and the pan was placed in the furnace, where the exact sample weight was determined. The sample was heated to 600 °C in an inert nitrogen atmosphere at the rate of 5 °C/min. Sample weight differences were automatically

Table 1. Proximate Composition (Percent) of Whole Herring, Herring Byproducts, and Arrowtooth Flounder Fillet[a]

component	WH	HB	HH	HG	AF
protein	14.5 ± 0.1b	16.9 ± 0.5a	13.1 ± 0.3c	18.0 ± 0.5a	17.1 ± 0.6a
fat	8.8 ± 0.5b	6.6 ± 0.7c	10.9 ± 0.6a	3.4 ± 0.2e	5.0 ± 0.6d
moisture	73.9 ± 0.6bc	74.4 ± 0.1b	72.4 ± 0.2c	77.5 ± 0.8a	77.4 ± 1.1a
ash	3.0 ± 0.2b	2.0 ± 0.2c	3.9 ± 0.3a	1.4 ± 0.0d	1.1 ± 0.1d

[a] Values are means ± SD of triplicate determinations. Means with the same letter in each row are not significantly different ($p > 0.05$). WH, whole herring; HB, herring body; HH, herring head; HG, herring gonad (male testes); AF, arrowtooth flounder fillet.

Table 2. Proximate Composition and Yield (Percent) of Fish Protein Powders from Whole Herring, Herring Byproducts, and Arrowtooth Flounder Fillet[a]

component	WHP	HBP	HHP	HGP	AFP
protein	76.2 ± 0.4b	73.4 ± 0.8c	73.9 ± 0.7c	63.0 ± 0.4d	81.4 ± 0.4a
fat	3.9 ± 0.4b	3.6 ± 0.3b	4.0 ± 0.4b	11.7 ± 0.7a	3.0 ± 0.04b
moisture	5.3 ± 0.04d	5.5 ± 0.3d	6.7 ± 0.2c	9.0 ± 0.2b	10.7 ± 0.4a
ash	14.8 ± 0.3c	17.7 ± 0.3a	15.6 ± 0.9bc	16.6 ± 0.5ab	4.8 ± 0.2d
yield	4.8 ± 0.3c	5.9 ± 0.4b	4.7 ± 0.4c	3.1 ± 0.2d	7.1 ± 0.6a

[a] Values are means ± SD of triplicate determinations. Means with the same letter in each row are not significantly different ($p > 0.05$). WHP, whole herring protein powder; HBP, herring body protein powder; HHP, herring head protein powder; HGP, herring gonad protein powder; AFP, arrowtooth flounder protein powder.

recorded every 0.5 s. Data were analyzed and plotted using the TA Universal Analyzer software. Graphs were normalized based on the sample weight.

Statistical Analysis. Mean values from the three separate experiments were analyzed. The statistical significance of observed differences among treatment means was evaluated by analysis of variance (ANOVA) (SAS version 8.2, SAS Institute Inc., Cary, NC), followed by the post hoc Tukey's studentized range test (*15*).

RESULTS AND DISCUSSION

Proximate Composition. The proximate composition of whole herring, herring byproducts, and arrowtooth flounder fillets used as raw materials for the FPP preparation is given in **Table 1**. The protein contents of WH, HB, HH, HG, and AF were 14.5, 16.9, 13.1, 18.0, and 17.1%, respectively. The fat content of HH (10.9%) was greater ($p < 0.05$) than that of WH, HB, HG, and AF.

The proximate composition of the FPP samples, prepared from WH, HB, HH, HG and AF, is shown in **Table 2**. AFP had the highest protein content of 81.4%, whereas HGP had the lowest protein content of 63.0%. Among the herring FPP samples, WHP had the highest protein content (76.2%). The protein contents of the herring FPP samples (**Table 2**) were similar to those (77−87.9%) reported by Hoyle and Merritt (*9*) and Liceaga-Gesualdo and Li-Chan (*16*) for dried hydrolyzed herring components. The fat content of HGP (11.7%) was much higher ($p < 0.05$) than that of other FPP samples, which ranged from 3.0 to 4.0%. The ash content (4.8%) of AFP was significantly ($p < 0.05$) lower than that (14.8−17.7%) of the herring FPP samples. Other investigators (*16*−*18*) have reported ash contents of 9−22% for fish protein isolate.

The FPP extraction yield was calculated by determining the weight of dried FPP as a percentage of the total wet weight of fish used (**Table 2**). The FPP yield ranged from 3.1 to 7.1%. The AFP extraction yield (7.1%) was significantly ($p < 0.05$) higher than that (3.1−5.9%) of all herring FPP samples. The lower extraction yield of herring FPP protein was likely due to insoluble proteins, nonprotein nitrogen, and nonprotein components associated with the bones and skins. In addition, protein hydrolysis caused by endogenous proteolytic enzymes (*3*) present in the arrowtooth flounder muscle may have contributed to the higher yield of AFP. The yield (3.1−5.9%) of our herring FPP samples is similar to those (3.6−5.5%) previously reported by Hoyle and Merritt (*9*). When calculated on a dry weight basis of fish used (**Table 1**), the yields of FPP are 18.3% for WHP, 23.1% for HBP, 16.8% for HHP, 13.6% for HGP, and 30.6% for AFP. A number of variables influence solubility and protein extraction efficiency from fish tissues, including concentration and particle size of suspended tissues, extraction time, temperature, pH, and the type and concentration of salts used for extraction (*19*). Also, the extraction yield depends on the freshness of the raw materials. In addition, different tissues contain different amounts of specific proteins; for example, fillet has an abundance of actin and myosin, and skin contains a high level of collagen. It has been reported that 85−95% of the protein of fish muscle may be extracted by blending fish muscle with 0.5−1.0 M neutral salt solutions (*20*). In this study protein was extracted from the fish tissues with water.

Table 3. Color $L^*a^*b^*$ Values of Fish Protein Powders from Whole Herring, Herring Byproducts, and Arrowtooth Flounder Fillet[a]

sample	L^*	a^*	b^*
WHP	82.8 ± 2.5b	3.5 ± 0.3b	12.5 ± 0.5d
HBP	81.4 ± 1.2b	3.7 ± 0.2b	15.8 ± 0.5b
HHP	68.5 ± 0.9c	5.7 ± 0.5a	14.1 ± 0.6c
HGP	67.4 ± 1.6c	5.8 ± 0.3a	27.9 ± 0.9a
AFP	86.3 ± 2.6a	2.2 ± 0.2c	2.8 ± 0.5e

[a] Values are means ± SD of triplicate determinations. Means with the same letter in each column are not significantly different ($p > 0.05$). WHP, whole herring protein powder; HBP, herring body protein powder; HHP, herring head protein powder; HGP, herring gonad protein powder; AFP, arrowtooth flounder protein powder.

Color and Molecular Weights. The FPP samples were light yellow in color (**Table 3**). HGP and HHP were darkest ($p < 0.05$), with L^* values of 67.4 and 68.5, respectively. AFP was the lightest ($L^* = 86.3$) and least yellowish ($b^* = 2.8$). HGP was most yellowish, with the b^* value of 27.9. Descriptive sensory properties of the FPP samples were not evaluated in this study; however, a slight fish odor and taste, accompanied by a metallic taste, were apparent in the samples. Residual fish lipid in the FPP samples may have caused the fish-like and metallic odors. A three-phase decanter could be used in a larger scale production of FPP to reduce the fat content of the FPP samples. The herring FPP samples had discrete protein bands with molecular weights ranging from 130,000 to <13,000 Da (**Figure 1**), although some degraded matter was present. These patterns are consistent with soluble proteins, some of which could be partially degraded. The gel profiles for WHP, HHP, and HBP did not contain bands at 210 kDa, consistent with the absence of myosin heavy chains. This observation is consistent with myofibrillar protein precipitating during the initial heating step and removed in the centrifugation step. The AFP samples had a smear of low molecular weight protein material below 13 kDa, indicating the protein was hydrolyzed. Hydrolysis of the AFP was due to a high level of proteolytic activity found in the arrowtooth flounder muscle. During the heating process the proteolytic enzymes in the arrowtooth flounder muscle are activated, and proteolytic degradation may have occurred (*3*).

Amino Acid Analysis and Mineral Content. The essential amino acid content of the FPP samples was compared with the

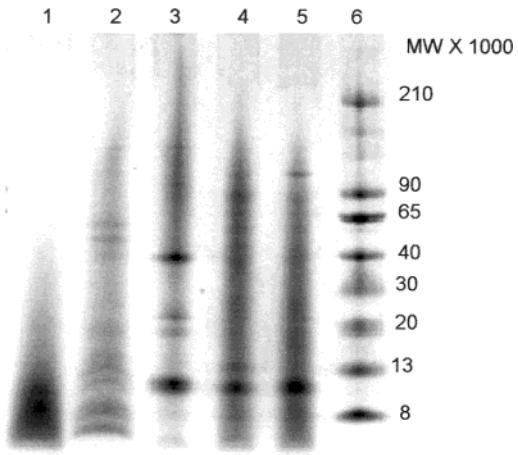

Figure 1. SDS-Tricine/polyacrylamide gel electrophoresis profiles of the fish protein powders and SDS marker: lane 1, APF; lane 2, HGP; lane 3, HBP; lane 4, HHP; lane 5, WHP; lane 6, SDS marker (8–210 kDa). AFP, arrowtooth flounder protein powder; HGP, herring gonad protein powder; HBP, herring body protein powder; HHP, herring head protein powder; WHP, whole herring protein powder.

Table 4. Amino Acid Composition of Fish Protein Powders from Whole Herring, Herring Byproducts, and Arrowtooth Flounder Fillet[a]

amino acid	WHP	HBP	HHP	HGP	AFP	EAA[b]	EAA[c]
hydroxyproline	20.4	28.5	33.5	3.1	5.9		
cysteine	8.4	6.4	8.8	15.7	12.2		
aspartic acid	78.5	75.0	77.0	56.6	99.2		
threonine[d]	34.8	29.3	34.8	34.7	38.2	9	43
serine	41.5	40.3	45.0	43.5	41.6		
glutamic acid	169.7	130.1	142	213.5	204.3		
proline	50.8	56.0	67.1	41.3	26.4		
glycine	105.5	144.1	133.3	163.8	50.9		
alanine	81.9	94.6	80	54.3	63.0		
valine[d]	37.4	29.3	36.2	35.4	39.6	13	55
methionine[d]	29.2	28.8	30	20.9	30.0	17[e]	42[e]
isoleucine[d]	26.0	22.0	23.3	24.2	33.0	13	46
leucine[d]	72.0	57.8	59.9	48.7	90.4	19	93
tyrosine	16.4	14.3	18.0	21.6	19.5		
phenylalanine[d]	30.8	37.7	34.2	27.8	28.2		
histidine[d]	17.0	28.0	15.0	17.3	20.8	16	26
lysine[d]	94.6	91.7	73.0	51.3	117.4	16	66
arginine	82.7	73.3	84.0	121.1	76.0		
TEAA	341.8	324.6	306.4	260.3	397.6		
TAA	997.6	987.2	995.1	994.8	996.6		
TEAA/TAA (%)	34.3	32.9	30.8	26.2	39.9		

[a] Data are expressed as mg of amino acid per g of protein. Tryptophan was not determined. TEAA, total essential amino acids; TAA, total amino acids; WHP, whole herring protein powder; HBP, herring body protein powder; HHP, herring head protein powder; HGP, herring gonad protein powder; AFP, arrowtooth flounder protein powder. [b] Suggested profile of essential amino acid requirements for adult humans by FAO/WHO (*21*). [c] Suggested profile of essential amino acid requirements for infants by FAO/WHO/UNU (*22*). [d] Essential amino acids. [e] Methionine + cysteine.

recommendations made by FAO/WHO (*21*) for adult humans and by FAO/WHO/UNU (*22*) for infants. AFP and herring FPP samples (except HHP for histidine) exceeded the essential amino acid requirements for adult humans (**Table 4**). The lysine content of all FPP samples (except HGP) exceeded the essential amino acid requirements for infants; however, other essential amino acids of the FPPs were generally below the infant requirements. All essential amino acids (except phenylalanine and histidine) were higher in AFP than in the herring FPP samples. The hydroxyproline contents of AFP and HGP were much lower than those of other herring FPP samples. The higher values of hydroxyproline in WHP, HBP, and HHP indicate higher levels of connective tissue proteins such as gelatin in these samples.

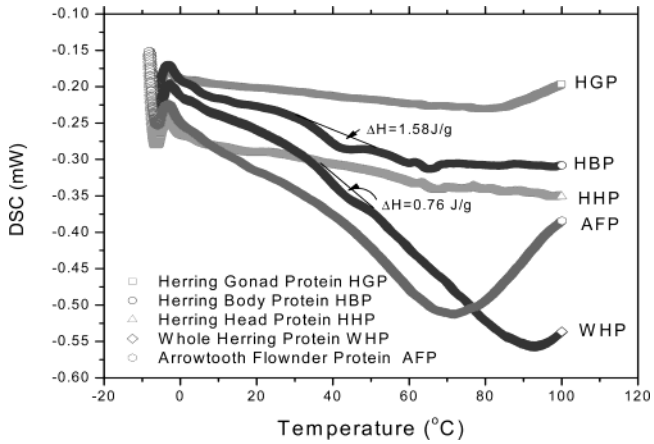

Figure 2. DSC thermograms of fish protein powders. WHP, whole herring protein powder; HBP, herring body protein powder; HHP, herring head protein powder; HGP, herring gonad protein powder; AFP, arrowtooth flounder protein powder.

The ratio of the total essential amino acids to the total amino acids (TEAA/TAA) was higher in AFP than in the herring and herring byproduct protein powders.

The FPP samples were rich in K, Na, P, S, Mg, and Ca (**Table 5**). All FPP samples had relatively low calcium to phosphorus ratio values, which is typical for products lacking bone. The zinc content of HHP (39.5 ppm) was significantly higher than that of other FPP samples.

Functional Properties. Solubility is one of the most important physicochemical and functional properties of protein concentrates (*23*, *24*). Nitrogen solubility values for the FPP samples and the two reference proteins, egg albumin and soy protein concentrate (SPC), are shown in **Table 6**. The nitrogen solubility values of AFP, WHP, HBP, and HHP were >78%. The different solubilities observed among the FPP samples may be attributed to denaturation that may have occurred during the FPP preparation. Among herring FPP samples, HHP had the highest solubility (87.2%), although it was not significantly different from those of AFP and WHP. The nitrogen solubility value of egg albumin was not significantly different from those of AFP and HHP. SPC was less soluble (9.8%) than were all FPP samples. The high nitrogen solubility of FPPs indicates potential applications in formulated food systems by providing an attractive appearance and a smooth mouthfeel to the product (*25*).

Generally fish proteins are less soluble around neutral pH, except at very low ionic strength (*26*). Denaturation of proteins during processing could have contributed to decreased solubility by unfolding of the protein and exposing the hydrophobic groups located in the interior part (*27*). Solubility values (89.7−93.1%) of enzymatically hydrolyzed herring muscle protein (*9*) were higher than those of FPP samples in this study (**Table 6**). The higher solubility of hydrolyzed protein is due to smaller peptides with increased availability of polar residues to form hydrogen bonds with water.

Emulsifying capacity (EC) of the FPP samples was compared with that of egg albumin and SPC (**Table 6**). EC of all FPP samples was lower than that of egg albumin but slightly higher than that of SPC ($p < 0.05$). HGP had the highest EC among the FPP samples. The emulsifying stability (ES) of all FPP samples (57−67%) was lower ($p < 0.05$) than that of egg albumin (72%). ES of SPC (62%) was similar to that of HBP, HHP, and HGP. ES of all herring FPP samples was greater than that of AFP. The ES value of 52−61% was reported for

Table 5. Mineral Content (Parts per Million) of Fish Protein Powders from Whole Herring, Herring Byproducts, and Arrowtooth Flounder Fillet[a]

mineral	WHP	HBP	HHP	HGP	AFP
Al	1.1 ± 0.6c	4.8 ± 1.2bc	9.8 ± 2.7b	8.5 ± 2.3b	41.7 ± 0.9a
B	5.2 ± 0.1b	3.5 ± 0.4b	12.1 ± 0.5a	4.9 ± 2.2b	2.4 ± 0.6b
Ca	1375 ± 51.8ab	1097 ± 30.8bc	1457 ± 86.8ab	852 ± 152c	1624 ± 319a
Cu	7.0 ± 0.3a	2.6 ± 0.4b	5.9 ± 0.4a	5.9 ± 0.5a	3.1 ± 0.7b
Fe	18.9 ± 0.7b	15.5 ± 2.5b	107.7 ± 31a	99.2 ± 16.2a	21.8 ± 8.6b
K	36790 ± 384.7c	49737 ± 621.9a	29814 ± 1861d	43389 ± 2035b	44409 ± 1958b
Mg	2492 ± 19.1a	2630 ± 41a	2041 ± 118.2b	1978 ± 90b	2629 ± 111a
Mn	1.0 ± 0.1b	0.48 ± 0.11b	0.6 ± 0.2b	1.6 ± 0.4b	6.8 ± 1.5a
Na	20530 ± 122.4b	17054 ± 222.3b	31892 ± 2682a	30333 ± 1886a	5735 ± 325c
P	22269 ± 294b	26210 ± 360a	14125 ± 772c	25425 ± 738a	22256 ± 598b
S	11090 ± 169c	9919 ± 144d	13234 ± 424b	14543 ± 285a	6371 ± 173e
Zn	15.5 ± 1.8b	14.0 ± 1bc	39.5 ± 4.1a	10.6 ± 1.3bc	9.2 ± 1.5c

[a] Values are means ± SD of triplicate determinations. Means with the same letter in each row are not significantly different ($p > 0.05$). WHP, whole herring protein powder; HBP, herring body protein powder; HHP, herring head protein powder; HGP, herring gonad protein powder; AFP, arrowtooth flounder protein powder.

Table 6. Functional Properties of Fish Protein Powders from Whole Herring, Herring Byproducts, and Arrowtooth Flounder Fillet[a]

sample	nitrogen solubility (%)	emulsifying capacity (mL of oil/200 mg of protein)	emulsifying stability (% emulsified)	fat absorption (mL of oil/g of protein)
AFP	87.0 ± 1.9ab	96 ± 4.8c	57.3 ± 2.1d	8.4 ± 0.2c
WHP	83.4 ± 3.9b	103 ± 6.9c	67.3 ± 1.1b	9.6 ± 0.1b
HBP	78.1 ± 1.2c	99 ± 1.2c	65.6 ± 0.9bc	11.5 ± 0.8a
HHP	87.2 ± 4.5ab	104 ± 5.3c	66.4 ± 1.7bc	6.7 ± 0.2d
HGP	63.4 ± 0.9d	132 ± 2.8b	64.5 ± 0.9bc	3.9 ± 0.1f
egg albumin	89.8 ± 3.5a	327 ± 18.1a	72.3 ± 3.4a	5.1 ± 0.8e
SPC	9.8 ± 0.1e	76 ± 6.8d	62.2 ± 4.2c	3.6 ± 0.3f

[a] Values are means ± SD of triplicate determinations. Means with the same letter in each column are not significantly different ($p > 0.05$). WHP, whole herring protein powder; HBH, herring body protein powder; HHP, herring head protein powder; HGP, herring gonad protein powder; AFP, arrowtooth flounder protein powder; SPC, soy protein concentrate.

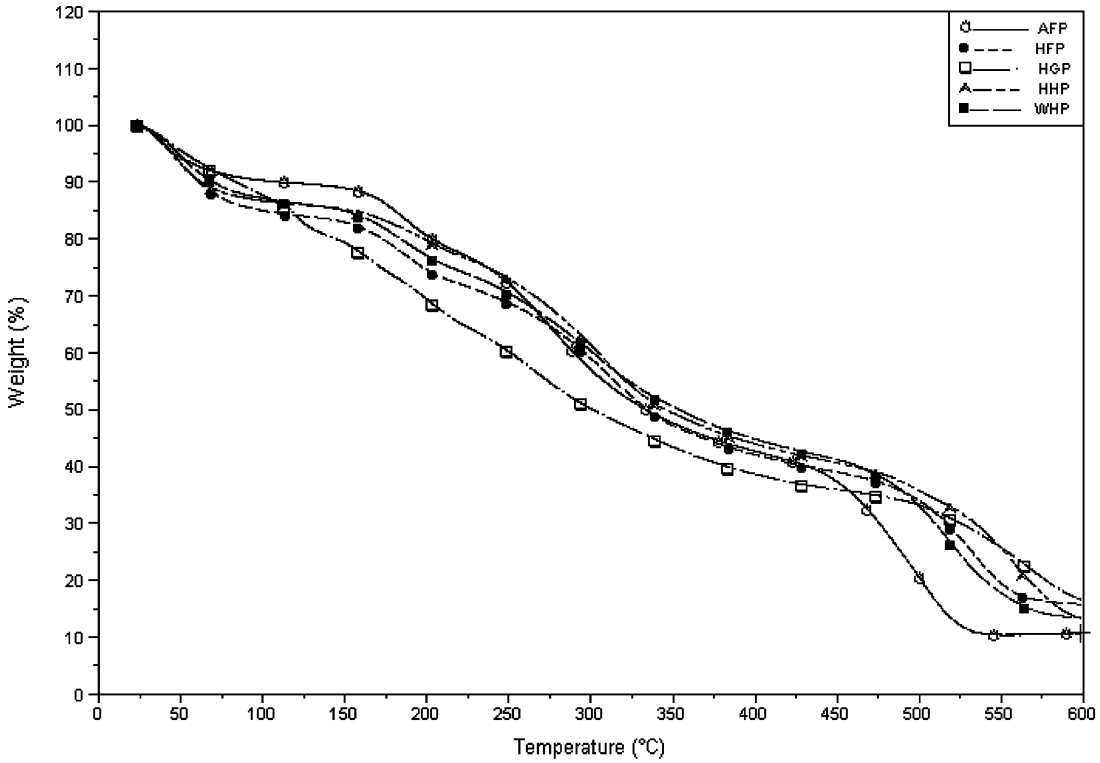

Figure 3. TG thermograms showing weight loss curve for fish protein powders. WHP, whole herring protein powder; HBP, herring body protein powder; HHP, herring head protein powder; HGP, herring gonad protein powder; AFP, arrowtooth flounder protein powder.

enzymatically hydrolyzed Atlantic salmon (*Salmo salar*) muscle protein (*28*). A positive correlation between surface activity and peptide length was reported (*29*), and a peptide should have a minimum length of 20 residues to possess good emulsifying and interfacial properties (*30*). Protein solubility and hydrophobicity (*31*) and peptide lengths (*29*) all play an important role in emulsion properties. Globular protein tends to form a highly elastic adsorbed layer (*32*) with increased viscosity that contributes to the emulsion stability. Proteins that retain their tertiary structure at the interface will maintain an extensive

intermolecular network (protein−protein interaction) that forms a more stable emulsion.

Fat binding/absorption capacity is an important functional characteristic of ingredients used in the meat and confectionery industries. As shown in **Table 6**, HBP had the highest fat absorption capacity (11.5 mL/g of protein), whereas SPC had the lowest (3.6 mL/g of protein). With the exception of HGP, all of the FPP samples exhibited a greater ability ($p < 0.05$) to bind soybean oil than did egg albumin and SPC. The lower fat binding capacity of HGP may be related to its bulk density. We observed that HGP and SPC did not well disperse in the soybean oil, and both had the tendency to clump. Kristinsson and Rasco (*28*) reported fat absorption capacity values ranging from 2.86 to 7.07 mL of oil/g of protein for Atlantic salmon protein hydrolysates. Bulk density of the protein (*23*), degree of hydrolysis (*28*), and specificity of the hydrolyzing enzyme (*33*) affect the ability of hydrolysates to bind fat.

WHP could be cheaper to produce because the cost involved for separating parts is absent. Analysis of the functional properties for the protein powders from WH (male and spent female herring) and AF indicates that these two protein powders are comparable in many respects to industrial proteins such as soy protein. These protein powders could be used as emulsifier and gelling agents.

Thermal Analysis. During the extraction and preparation process, fish protein powders are subjected to temperature changes, which may alter their physical state. The most commonly occurring phase transition in protein is denaturation, which can alter the properties of protein and thus the quality of final products. The DSC thermograms of the FPP samples are shown in **Figure 2**. The DSC thermogram for HBP and WHP showed a single small endothermic transition with total enthalpy, ΔH_{total}, values of 1.58 and 0.76 J/g, respectively. Endothermic transitions were not found for HGP, HHP, and AFP. Although the results indicate a small transition in HBP and WHP, all protein powders were subjected to an initial 85 °C heating step, which would have denatured proteins in the FPP.

Knowledge of the thermal decomposition of the FPP samples can be used to improve their stability and functional properties. A TG analyzer is a balance, which measures changes in weight as a function of changing temperatures. A series of the TG thermogram of the FPP samples is shown in **Figure 3**. Between 0 and 600 °C, weight loss of FPP samples increased with increasing heating temperatures and the mass losses were slightly different among the FPP protein powders. Four weight-loss temperature regions were identified for WHP, HBP, HHP, and AFP and six regions for HGP. Significant weight reduction occurred between 450 and 600 °C for all FPP samples. The TG curves indicated the thermal stability in the following order: HGP > HBP > WHP > HHP > AFP. The mass losses at 600 °C were 83.5, 84.2, 86.5, 86.9, and 89.3% for HGP, HBP, WHP, HHP, and AFP, respectively. The differences may be due to the presence of other components that interact with protein in powders such as phospholipids, complexed metals (notably iron, calcium, and magnesium minerals), free fatty acids, and peroxides and their breakdown products. The presence of those components reduces the effectiveness of heat transfer to protein powders and, thus, the mass losses of protein powders.

The results indicate that FPP can be potentially used as functional ingredients and nutritional supplements. The emulsifying capacity, emulsifying stability, fat absorption capacity, and nitrogen solubility for WHP were higher than those for SPC. AFP showed a higher emulsifying capacity, emulsifying stability, and fat absorption capacity than SPC. The FPP powder is a good source of high-quality fish protein and could compete industrially with other protein powders such as soy protein isolate and egg albumin. The functional properties of the FPP could be used as emulsifiers and gelling agents. This study identifies opportunities to develop value-added products from Alaska fish-processing byproducts.

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